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Excluding myeloma diagnosis using revised thresholds for serum free light chain ratios and M-protein levels

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In Western Europe in 2016 there were 35,000 new cases of and 22,000 deaths from multiple myeloma.(1) Myeloma is one of the worst cancers for delays in diagnosis and half of patients saw their primary physician three or more times before referral to a haematologist.(2) England population data show a third of 22,249 newly diagnosed myeloma patients presenting as emergencies and having the worst 12 month survival at just 61%.(3) Myeloma is suspected when patients present with one or more features of myeloma related end organ damage including anaemia, skeletal disease, renal impairment and immunodeficiency but any of these are more likely caused by much commoner diseases. Myeloma is rare in primary care consultations and the symptoms are non-specific, common and of low predictive value (Odds Ratios OR $\leq$3).(4, 5)

Critical to diagnosis of myeloma is testing serum for the presence of an M-protein (paraprotein) plus serum and/or urine testing for monoclonal free light chains (FLC). Absence of an M-protein and abnormal serum FLC ratio (SFLCR) is only found in the rare non-secretory myeloma, and therefore with close to 100% sensitivity for diagnosis of myeloma, encouraging widespread use of these simple blood tests may reduce delays in myeloma diagnosis.(6) However, ordering these tests is tempered because they also reveal the hundred times more common condition Monoclonal Gammopathy of Undetermined Significance (MGUS).(7) In clinical practice the majority of M-proteins and abnormal SFLCRs derive from MGUS plasma cell clones or are small SFLCR abnormalities caused by conditions unrelated to neoplastic plasma cells including kidney disease, inflammation and infection. Myeloma arises in an age range where these conditions are common and there is a need to better define SFLCR reference ranges in these groups of patients.(8-10) MGUS is not usually clinically significant and not the cause of the patients presenting illness but requires differentiation from myeloma and this can be difficult.(11) Particularly in primary care and other specialties there may be unnecessary rapid referrals to haematologists, inappropriate

testing (imaging or bone marrow biopsies), and associated patient anxiety. This is a significant problem in the UK where patients are frequently referred unnecessarily as urgent suspected cancer cases and contributes to gross inefficiency within healthcare systems that are struggling with capacity. There is a lack of guidance for non-haematologists in interpretation of abnormal test results in this setting. It would be useful to have evidence based guidelines on laboratory reporting of M-protein levels and SFLCR that enabled primary care and other specialty doctors to decide which patients to refer urgently to haematology for suspected myeloma.

We examined the use of different M-protein level thresholds combined with different SFLCR ranges to distinguish myeloma from MGUS without loss of sensitivity for identifying patients that need urgent referral for myeloma diagnosis and assessment. Central laboratory SFLCR and M-protein concentrations in 3177 newly diagnosed myeloma patients from UK clinical trials(12, 13) and 711 MGUS cases were analysed.(7)

The distribution of M-protein levels for myeloma patients with an IgG or IgA M-protein (accounting for 84% of all myeloma patients) are shown in Figure 1. IgG patients had significantly higher M-protein levels (median 37 g/L, range 0.80–129 g/L) compared to IgA patients (median 34.5 g/L, range 0.2–109 g/L), $p < .001$ likely to reflect the longer serum half-life of IgG. A cut-off of 30g/L is used to separate MGUS from smouldering (asymptomatic) myeloma. In this large cohort of newly diagnosed myeloma patients that required anti-myeloma therapy 33% of IgG patients and 43% of IgA patients had M-proteins <30 g/L. 5% of IgG patients and 11% of IgA patients presented with an M-protein level < 10 g/L, below the threshold recommended for accurate assessment of M-protein response to therapy.

Patients were classified as having a normal or abnormal SFLCR according to the diagnostic ratio reference range (NRR) of 0.26–1.65,(14) as per the International Myeloma Working Group (IMWG) guidelines for diagnosis of all plasma cell dyscrasias.(15) To investigate alternative RRs, all trial patients' SFLCR at myeloma diagnosis were rank ordered. Five percent of myeloma patients had a SFLCR within the NRR. SFLC ranges were then extended outwards to encompass 10% then 15% of all patients at diagnosis. Two thirds of myeloma patients have κ FLC and one third λ FLC. To reflect this, the extended kappa lambda RRs were placed above 1.65 to add two thirds of the additional patients as kappa and below 0.26 to add one third of additional patients as lambda. This generated extended reference range 1 (ERR1 [0.15–3.36]) and extended reference range 2 (ERR2 [0.08-7.41]).

24 patients (0.8% of total 3177 patients) were non secretors defined by immunofixation negative blood and urine and SFLCR within the NRR; these were excluded from the analysis. Patients with secretory disease (m-protein and/or FLC, n = 3153) were stratified by a normal SFLCR according to each of the three reference ranges (NRR 0.26–1.65; ERR1 0.15–3.36; ERR2 0.08–7.41) and when using the SFLCR test alone these failed to identify 5.2%, 10% and 15% of all newly diagnosed myeloma patients respectively (Table 1). For the 436 patients who had an abnormal SFLCR and no M-protein (light chain only (418) and oligosecretory (18) patients), ERR1 missed 2 patients and ERR2 missed 5 patients. For the 2,717 patients with an IgG/A/M or D M-protein, the SFLCR test alone missed 4.7%, 9.2% and 14.4% for the ranges NRR, ERR1 and ERR2, respectively.

Patients secreting an M-protein were then sub-divided into those with/without an M-protein level of < 5 g/L, < 10 g/L and < 15 g/L (Table 1). The use of the three SFLCR ranges combined with these specified M-protein levels greatly enhanced sensitivity for the identification of new myeloma. Using the NRR, just 0.4–0.9% of all patients were missed

when using both biomarkers across the different M-protein cut-points. In the same way the broader ERR1 missed 0.5–1.6% and the broadest ERR2 missed 0.5–2.1% of all new myeloma patients. For the ease of use in clinical practice, a simplified ratio may be most appropriate. We propose 0.1–7.0, which, as shown in Table 1, yielded very similar percentages and numbers to EER2. This data demonstrates that, combined with an M-protein threshold of 10 g/L, significantly widening the SFLR reference range from the NRR of 0.26–1.65 to 0.1–7.0 only decreased sensitivity for identification of new myeloma by 0.5%.

We next applied these extended SFLCR ranges and M-protein thresholds to a cohort of 711 MGUS patients (484 IgG, 109 IgA and 118 IgM), to assess how this strategy could be used to exclude MGUS patients from urgent in depth myeloma investigations. As shown in Table 2, use of the SFLCR on its own excluded 53.9% (NRR) up to 88.9% (EER2) of MGUS patients. Combining SFLRs with the three M-protein cut-points (<5, <10, <15 g/L) then 68.8%, to 90.1% MGUS patients were excluded using the NRR and 89.5% to 95.5% excluded using the simplified SFLCR range of 0.1 – 7.0

The use of the SFLCR range of 0.1–7.0 in combination with an M-protein threshold of 10 g/L included 97.9% of myeloma cases and excluded 93.4% of MGUS cases. The 2.1% of myeloma patients missed (n = 66) included 0.8% of non-secretors. Using other commercially available assays, such as N Latex (Siemens, Germany) and Seralite (Abingdon Health, UK) then these greatly widened SFLR ranges are likely to provide similar sensitivity and specificity for distinguishing myeloma from MGUS but this needs to be tested.

A similar process combining the use of M-protein levels plus SFLCR has provided a useful tool for risk stratification of MGUS patients for progression to myeloma.(7) Our proposed model adds an additional tool to aid clinicians in the differentiation of those patients at high risk that myeloma is the cause of their presenting illness versus those more likely to have

another disease causing there presenting illness with coincidental MGUS. This should of course not be used in isolation and should be in conjunction with clinical symptoms and other laboratory biomarkers. The use of this tool may enable the wider application of myeloma screening in patients presenting with features of myeloma related end organ damage. This may be important to attenuate the delays in myeloma diagnosis which negatively affects patient outcomes. In conclusion, applying an M-protein threshold of 10 g/L combined with a SFLCR range of 0.1-7 excludes 93.4% of MGUS cases and provides 97.9% sensitivity for detection of myeloma. This sensitivity is just 0.5% less than if using the SFLCR five-fold tighter range of 0.26–1.65 that has poor specificity for myeloma.

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Table 1. The percentage of myeloma patients who would be undetected at diagnosis according to serum free light chain κ:λ ratio ranges and M-protein level thresholds

	Percentage and number of patients missed if using FLC ratio alone to detect myeloma			Percentage and number of patients missed using both FLC ratio and m-protein level		
κ:λ ratio ranges	**All patients (N = 3177)**	**Light chain only (n = 436, 13.8%)**	**IgG/A/M/D (n = 2717, 86.2%)**	**M-protein < 5g/L (n = 122)**	**M-protein < 10g/L (n = 226)**	**M-protein < 15g/L (n = 336)**
Normal ratio range **(0.26–1.65)**	5.2% (172)	0	4.7% (148)	0.4% (12)	0.7% (23)	0.9% (29)
Extended ratio range 1 **(0.15–3.36)**	10% (319)	0.06% (2)	9.3% (293)	0.5% (16)	1.1% (35)	1.6% (49)
Extended ratio range 2 (**0.08–7.41)**	15% (478)	0.16% (5)	14.2% (449)	0.5% (16)	1.3% (40)	2.1% (65)
Proposed reference range **(0.1–7.0)**	13.8% (438)	0.13% (4)	13.0% (410)	0.5% (16)	1.2% (38)	2.0% (62)

Patients were classified as having a normal sFLC ratio according to the following reference ranges I) Normal range for SFLCR (0.26–1.65) II) an extended ratio range that encompassed 10% of all myeloma patients at disease presentation (0.15–3.36) III) an extended ratio range that encompassed 15% of all myeloma patients at disease presentation (0.08–7.41) IIII) A proposed reference range simplified for ease of us in clinical practice (0.1-7.0) For each of these SFLCR ranges, patients with a normal ratio were identified and then categorised according to myeloma type and in the shaded area M-protein level. Of 436 light chain only patients, 18 had oligosecretory myeloma (IFE negative in serum and urine but abnormal SFLCR and FLC levels sufficient to measure response to therapy) 24 patients (0.8% of total 3177 patients) were non secretors defined by immunofixation negative blood and urine and SFLCR within the NRR; these were excluded from the analysis: percentages are of total patients with secretory disease (n = 3153).

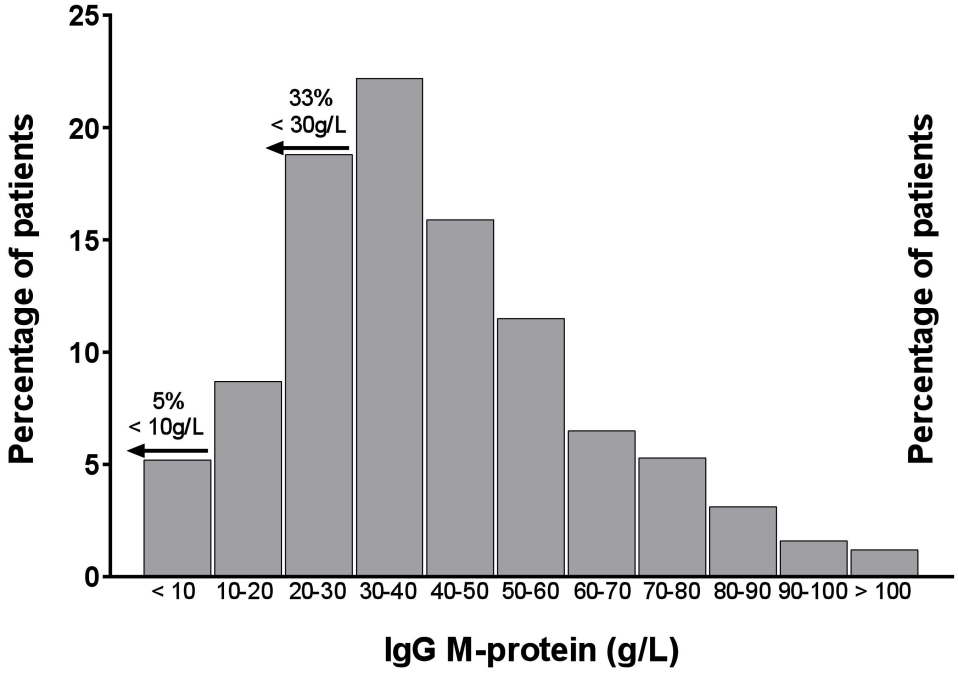
Table 2. The percentage of 711 patients with MGUS that would be excluded from further assessment for myeloma using the stated cut-offs for reference ranges for serum free light chain κ:λ ratio and ratio ranges combined with M-protein level thresholds

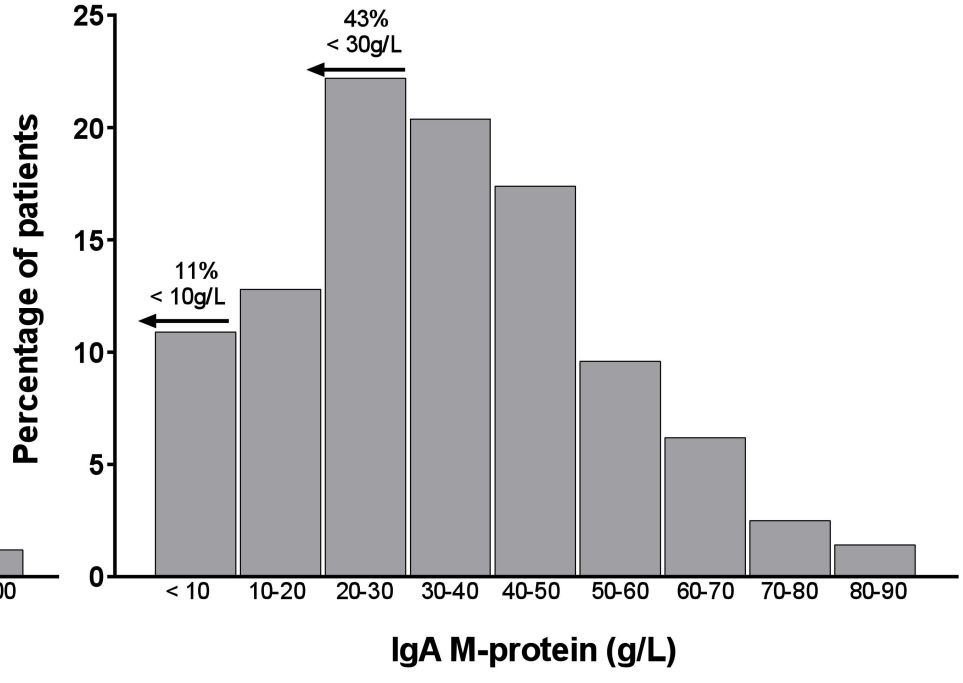
	% and number of patients excluded if using FLC ratio alone	% and number of patients excluded if applying sFLC ratio and m-protein level		
	All patients (n = 711)	**M-protein < 5g/L**	**M-protein < 10g/L**	**M-protein < 15g/L**
Normal ratio range **(0.26–1.65**)	53.9% (383)	68.8% (468)	81.2% (577)	90.1% (646)
Extended ratio range 1 **(0.15–3.36)**	77.8% (553)	81.7% (581)	88.6% (630)	93.2% (663)
Extended ratio range 2 (**0.08–7.41)**	88.9% (632)	90.7% (645)	94.2% (670)	96.2% (684)
Proposed reference range **(0.1–7.0)**	87.2% (620)	89.5% (636)	93.4% (664)	95.5% (679)

Patients were classified as having a normal SFLR according to the four different reference ranges and the percentage of patients that would be excluded from further assessment for myeloma is shown for each SFLCR range. The shaded area incorportates those with a normal ratio, then those with an abnormal ratio who can then be further excluded based on the three m-protein levels

Figure legends

Figure 1. Distribution of M-protein levels in IgG and IgA patients at trial entry in MIX and MXI clinical trials Data is shown for IgG (n =1894, left panel) and IgA (n =757, right panel) multiple myeloma patients at first diagnosis. A sizable proportion of these patients with symptomatic multiple myeloma presented with M-protein levels < 30 g/L, used as part of the criteria to separate MGUS (< 30g/L) and asymptomatic (smouldering) myeloma (> 30g/L) . A significant proportion of those patients presented with M-protein levels of < 10 g/L; the threshold for accurate assessment of response to therapy. Patients with IgG M-proteins had significantly higher M-protein levels compared to IgA patients (p <.001, Mann Whitney U-test.


Percentage of patients
25
20
15
10
5
0
33%
< 30g/L
5%
< 10g/L
< 10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
> 100
IgG M-protein (g/L)


Percentage of patients
25
20
15
10
5
0
43%
< 30g/L
11%
< 10g/L
< 10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
IgA M-protein (g/L)